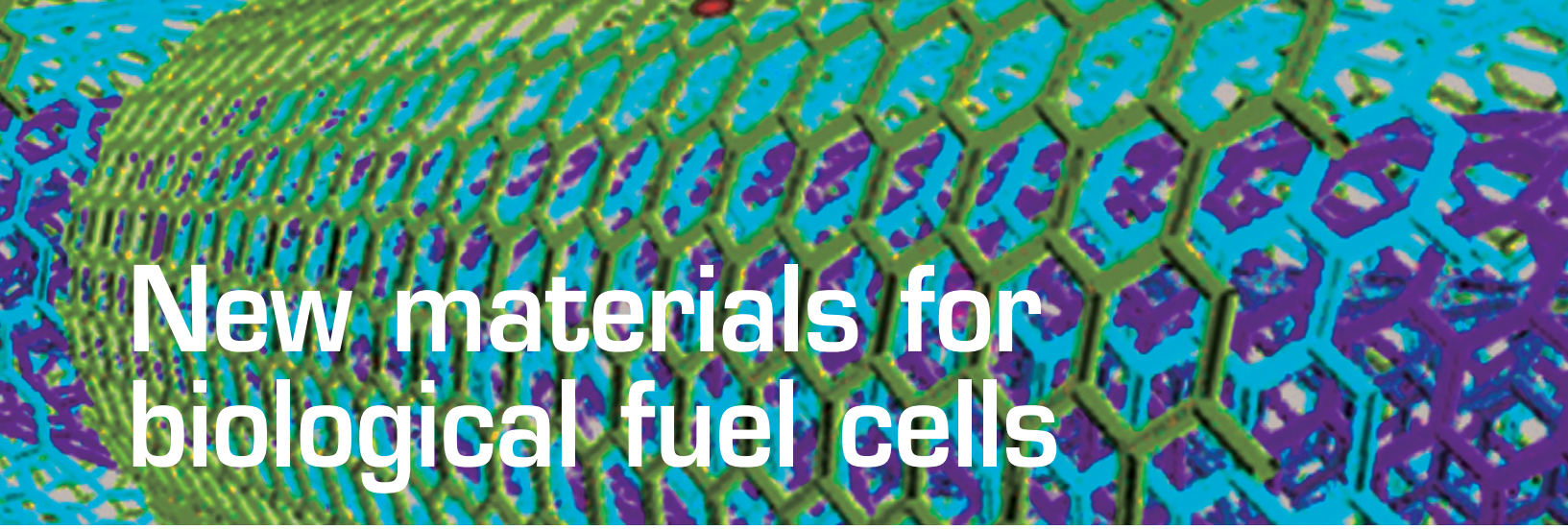

New materials for biological fuel cells

Major improvements in biological fuel cells over the last ten years have been the result of the development and application of new materials. These new materials include: nanomaterials, such as nanotubes and graphene, that improve the electron transfer between the biocatalyst and electrode surface; materials that provide improved stability and immobilization of biocatalysts; materials that increase the conductivity and surface area of the electrodes; and materials that aid facile mass transport. With a focus on enzymatic biological fuel cell technology, this brief review gives an overview of the latest developments in each of these material science areas and describes how this progress has improved the performance of biological fuel cells to yield a feasible technology.

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Over the last decade, there has been renewed interest in biological fuel cells[1-6]; a subset of fuel cells, where the cathode and/or anode catalysts are biocatalysts. This biocatalyst could be a living cell (microbial fuel cells) or a subcellular biological component (enzymatic or mitochondrial biological fuel cells). The first biological fuel cells were microbial and employed microbes at the anode to catalyze the oxidation of fuel[7]. Over the last century, the technology (frequently referred to as bio-electrochemical systems in the research community) has expanded to include microbial cathodes, with applications including wastewater treatment, underwater power, and the production of electrofuels[8-12]. Enzymatic biological fuel cells were first introduced in the 1960s, where oxidoreductase enzymes were used with mediators to catalyze the oxidation of amino acids, alcohol, and glucose at the anode of a fuel cell[13]. Continued development led to the use of enzymes at the cathode; where oxygen or peroxide is reduced to water in solution by an enzyme catalyst[14,15], or where oxygen is reduced directly in an air-breathing biocathode[16]. The choice of subcellular biocatalyst has since expanded to include organelles; with mitochondria, for example, used at the anode of pyruvate/air biological fuel cells[17].

Over the last decade, major improvements in biological fuel cells have actually been due to the incorporation of new materials, as well as the general move away from traditional H-cell designs toward engineered electrochemical cells. The specific improvements may be divided into

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four types of materials that will be discussed herein: (1) nanomaterials that improve the electron transfer from the biocatalyst to the electrode surface, (2) materials that offer greater stability and immobilization of the biocatalysts, (3) materials that increase the conductivity and surface area of the electrodes, and (4) materials that offer superior facile mass transport.

Nanomaterials that improve electron transfer

One key parameter of biological fuel cell performance is effective charge transfer; particularly in respect to enzymatic systems that rely on electron transfer between the electrode and the redox center of the enzyme. Electron transfer in biological fuel cells can occur via two general mechanisms: mediated electron transfer (MET) and direct electron transfer (DET).

MET occurs when the biocatalyst transfers electrons to a diffusible redox molecule or a redox polymer that in turn shuttles electrons between the biocatalyst and the electrode[4]. MET can be an efficient process when applied using reversible redox species with appropriate standard reduction potentials and defined concentrations of the mediator. There are drawbacks of MET, however, that must be rationalized in respect to fuel cell output and design. The primary limitation of diffusible mediators (more common in microbial biological fuel cells than enzymatic) is that most redox mediators are labile, imposing a limited lifespan that is further complicated by leaching during continuous operation of the cell. In addition, the half-cell potential is inherently limited by overpotential losses due to intermediate mediators, such as cofactors. The fuel cell design must also provide an absolute separation between half-cells in order to eliminate any crossover. Crossover is a common problem in all fuel cells, but can be circumvented by employing biological fuel cells that utilize selective, DET mechanisms by both anode and cathode catalysts.

DET processes eliminate many of the issues related to mediator use, since the electrons hop or tunnel directly between the biocatalyst and the electrode without any intervening *shuttle* molecules[4]. Designing bioelectrodes that carryout effective DET, however, is a challenge. In enzymatic fuel cells, for example, the enzyme must be arranged in such a way that its redox center is near the conductor electrode but not shielded by the enzyme structure, which acts as a non-conductive shell to limit electron transfer. Advances in nanomaterial synthesis and characterization are beginning to allow for specific control of the interaction; classed as the bio-nano interface. Defined protein assembly, combined with advances in bioelectrode architectures can significantly enhance DET processes and ultimately yield a realistic technology for small-scale biological fuel cells[18].

Various conductive and functionalized nanomaterials have been examined as bioelectrode materials[18,19]. The criteria for developing architectures that are suited to microbial and/or enzymatic systems are inherently different. Herein, in the interest of clarity and brevity, discussion is related primarily to materials architectures that facilitate effective electron transfer with proteins, although some overlap does exist with microbial systems and pertinent examples will be highlighted throughout. Development of microbial fuel cell electrodes typically requires a macroporous structure that is conducive to attachment of a large population of cells. Interestingly, microbes have been demonstrated to produce their own conductive nanowires (called pilli) that aid in the transfer of electrons via DET between the microbe and the electrode surface[20]. For enzymatic fuel cell electrodes, protein interaction and orientation at the nanoscale becomes more critical. As the aspect ratio of the nanomaterials approaches the molecular scale, the redox protein catalyst can establish a close association with the material, effectively decreasing the electron tunneling distance.

Primarily, the major enhancement in conductive interfaces for biomolecular electron transfer can be attributed to carbon-based materials; specifically: carbon black, carbon nanotubes (CNTs), and graphene.

Carbon black

Carbon black nanomaterials (CBN) are widely used to fabricate enzyme-functionalized electrodes as they possess characteristics well suited to a biological interface, i.e., a high porosity and relatively high surface area, coupled with high conductivity. Protein molecules adsorb onto CBN principally via hydrophobic-hydrophobic interactions: the interaction can be close enough to allow DET if the redox enzymes are preferentially orientated[21]. As such, multiple examples of CBN-based bioelectrodes are found in the literature. Ma *et al*. immobilized hemoglobin on standard carbon black powders (reportedly 30 – 100 nm diameter) and subsequently demonstrated direct oxidation and reduction of the heme-iron using cyclic voltammetry[22]. Kano and his colleagues at Kyoto University combined another model redox protein, cuperous oxidase (CueO) with Ketjen black to form a bioelectrocatalyst that could use atmospheric oxygen as a terminal electron acceptor and provide a modest cathodic current[23]. The addition of Ketjen black allowed current densities of the biocathode to increase from 3 – 4 mA/cm^2 for CueO on highly ordered pyrollytic graphite electrodes (HOPGE) to ~20 mA/cm^2 for Ketjen black incorporated electrodes. Similarly, increases in power density of 5 to 10-fold have been observed when Vulcan® XC-72 CBN are incorporated into PQQ-dependent alcohol dehydrogenase and PQQ-dependent glucose dehydrogenase-based biological fuel cells[18].

CBN are readily modified to create composites; emulsions of Teflon® and CBN, for example, are amenable to attachment on metallic, glassy carbon or other conventional electrode surfaces[24]. The combination of CBN and Teflon polymer provides a material matrix with the appropriate balance of hydrophobic-hydrophilic properties to yield a functional "electrolyte-carbon-air" tri-phase interface needed for gas diffusion electrodes (GDE)[25]. The CBN architecture has been demonstrated for the assembly of such GDE and recent advances have further improved upon the Ketjen-black based architectures[16,26,27].

Carbon nanotubes

The introduction of carbon nanotubes (CNTs) provided a novel tool for combining the bio-nano interface, due to inherent properties and

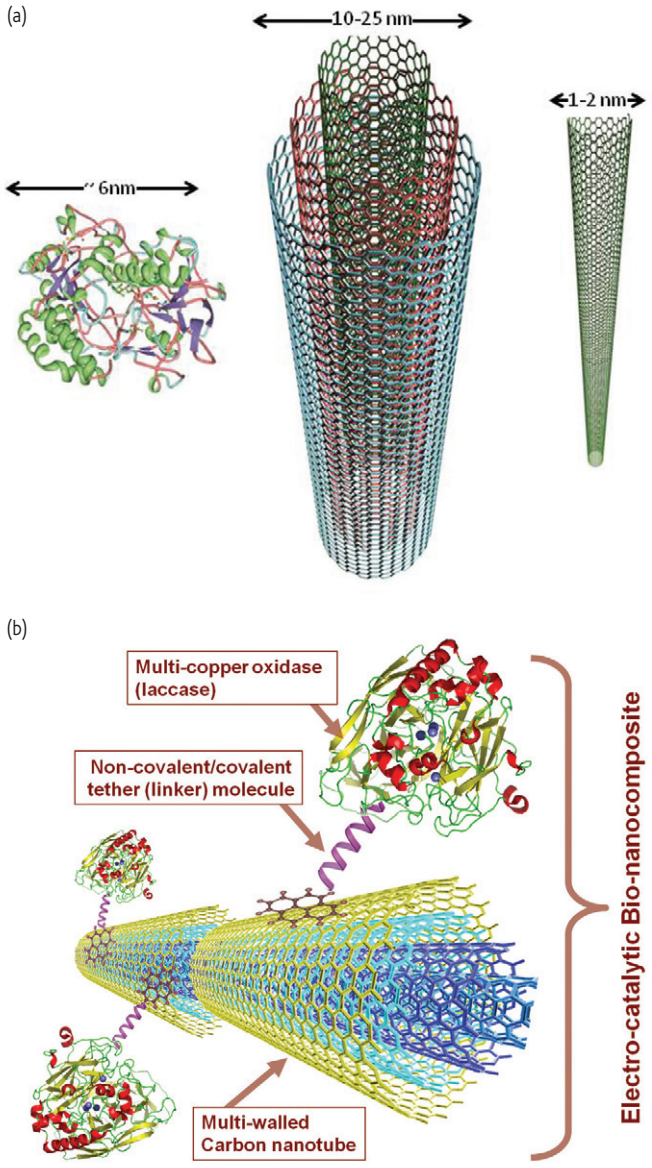

Fig. 1 (a) Relative dimensions of a glucose oxidase molecule and typical multiwall and single wall carbon nanotubes. (b) Schematic of the design of a bionanocomposite utilizing the phenol oxidase and multiwalled carbon nanotube material.

conductivity that provide a niche architecture well suited to fuel cells, sensors, and bio-electronics[28-30]. Both multi-wall carbon nanotubes (MWNTs) and single-wall carbon nanotubes (SWNTs), for example, have dimensions that are uniquely amenable to close physical, and then direct electronic interactions with redox proteins. A simple two-dimensional depiction of the materials and protein molecules gives a sense of scale and the potential interactions between redox enzymes and CNTs (Fig. 1). Further chemical functionalization of CNTs (e.g., amine-, carboxyl-, hydroxyl-groups) can be attained that maintain conductivity and provide additional sites for specific redox protein attachment. The typical dimensions of MWNTs would likely limit deep penetration toward the redox-center of the enzyme, instead the MWNT curvature is considered to be essentially "flat" from the perspective of the enzyme as a surface for attachment. Accordingly, there is an opportunity to take advantage of multiple surface moieties of a protein in order to link with the CNTs at specific and strategic tethering points. The options include both non-covalent (van der Waals, hydrophobic-hydrophobic, and ionic) and covalent interactions between unmodified and modified nanotubes[30]. By comparison, the dimensions of SWNTs may allow the conductive surface of the nanotube to physically access redox active sites, further decreasing the electron tunneling distance between the catalyst and electrode significantly.

Examples of CNT- and CNT-hybrid materials for bioelectrodes abound in the recent literature including materials architectures that are specifically advantageous for microbial fuel cells[18,31]. Many researchers, for example, have utilized CNTs to increase the surface area of electrodes, to improve the conductivity of porous scaffolds for biofilm growth, or to increase direct bioelectrocatalysis. For enzyme architectures, in the simplest case, a redox protein may contact a CNT surface by physisorption, with the protein adhering to the hydrophobic CNT surface, largely via van der Waals forces. Non-covalent interactions are somewhat labile, but have been used for establishing DET with a variety of redox enzymes[32-34]. The most common method of functionalizing CNTs is chemical oxidation to yield carboxylic acid groups at defect points on the CNT surface. The carboxyl groups can subsequently be activated by carbodiimide chemistry, which forms an unstable ester that will then react with accessible amino groups on the protein surface and form covalent amide bonds. The covalent link stabilizes the interactions and minimizes the distance between the protein and CNT surface, promoting electronic connectivity and DET. An example of this methodology is reported by Vaze *et al.*; with SWNTs-based electrodes and glucose oxidase (GOx) as the bioelectrocatalyst, the half-cell potential approached the theoretical redox potential for $FAD/FADH_2$ (−0.45V vs SCE) and current densities correlated to glucose concentration[34]. The results confirmed DET between the protein and electrode surface, and catalytic activity of the enzyme was retained[35]. There are caveats to this approach however, as the oxidation creates defects in the CNTs that decreases the conductivity of the material. Additionally, the short covalent link can result in steric constraints on the protein structure that reduce its catalytic activity[36]. Further experimental materials have combined CNTs with metal colloids and metal nanoparticles in an attempt to take advantage of the properties of each material for superior electrocatalysis[37,38].

Graphene

The emergence of graphene in research and its transition into a technologically viable material has provided a new dimension for bio-nanomaterials. Although it still remains to be determined if it is a practical material for constructing electrodes, initial studies demonstrate the potential for a less-explored, but electrochemically compelling material. Like CNTs,

graphene is amenable to covalent and non-covalent functionalization of the surface. The functionalization, if done correctly, does not appear to change the ballistic conductivity of the pristine graphene[39].

As such, the coupling of biocatalysts with graphene is beginning to receive interest in the literature. In one report, GOx was contacted with a graphene-glassy carbon electrode through simple physisorption and demonstrated catalytic activity and a characteristic DET response attributed to the flavin adenosine nucleotide (FAD) cofactor at –454 mV vs SCE[40]. The biopolymer chitosan, aids dispersion of various nanomaterials, including graphene, easing the formation of thin film electrodes. Again the model bioelectrocatalyst, GOx, was used to demonstrate the utility of the hybrid nanocomposite. GOx adsorbed to a chitosan/graphene thin film showed DET characteristics and a sensitive amperometric response to glucose concentrations (37.93 μA $mM^{-1}cm^{-2}$ versus 7.36 μA $mM^{-1}cm^{-2}$ for chitosan/MWNT). Using a chitosan architecture, the detection sensitivity increased two-fold compared to immobilization of GOx on graphene alone[41]. In other work, the same approach was demonstrated to combine cytochrome C, chitosan, and graphene on a glassy carbon electrode and DET was observed for the biocatalytic reduction of nitric oxide[42]. Further credence toward the utility of graphene-based bioelectrodes was provided by a side-by-side comparison against SWNT-based electrodes using GOx as the anode catalyst and billirubin oxidase as the oxygen reduction catalyst in the cathode. The current density of the assembled graphene-based fuel cell was double that observed for a SWNT architecture; moreover the power density with graphene was 3× greater than for a comparable SWNT fuel cell[43].

In parallel studies, graphene has recently found application in the development of microbial fuel cells. Li and co-workers, for example, have utilized the bacterium *Shewanella sp*. to reduce graphene for direct electron transfer[44].

Materials for stabilizing and immobilizing biocatalysts

As described above, the crux of effectively utilizing biomolecules in biological fuel cells is the effective orientation and interaction between an enzyme and a conductive transducer surface. Enzymes *ex situ* typically exhibit poor longevity, particularly when the local physiological environment pushes the optimal activity range of the enzyme. Particularly in bio-electronics applications, poor biocatalyst stability results in low power density and short lifetimes, because enzymes dissolved in solution at room temperature typically only have activity for a few hours and catalytic material is needed at the surface of the electrode to transport electrons efficiently[19]. Stabilization of the enzyme integrity is therefore essential to the efficiency and is typically achieved by various means of enzyme immobilization[45]. Immobilization serves to preferentially anchor the biomolecules in a manner that retains the native tertiary structure. Enzyme stabilization can also provide increased selectivity and may improve mass transfer kinetics. Immobilization strategies typically involve physical adsorption (primarily by electrostatic binding), entrapment in conducting polymer matrices, or covalent attachment to functionalized polymers[46]. Physical adsorption is attractive in its simplicity and although electrocatalytic activity can be retained, the power density is often low due to poor protein loading and leaching is a concern that limits lifetime. In contrast, covalent immobilization strategies provide superior electrocatalytic characteristics, but can sometimes hinder protein conformation[47]. In addition, the functional groups on the enzyme that are used for tethering should not be essential to catalysis or enzyme inactivation losses will occur.

Alternatively, methods of enzyme encapsulation can provide a means to stabilize proteins in a 'protective' environment by either trapping the protein, wiring the protein to the polymer backbone, or by specifically depositing enzymes within micellar pockets[48-50] (Fig. 2). Enzymes immobilized within the pockets of hydrophobically modified micellar polymers such as Nafion® and chitosan, for example, have been shown to effectively stabilize enzymes at electrode surfaces and promote operation lifetimes of more than two years[48].

A wide variety of redox catalysts can be stabilized by encapsulation during silica sol-gel formation[51–53]. Conductivity of the silica matrix can be achieved by co-immobilization of a conductive material, such as CNTs. The cationic protein, lysozyme for example, catalyzes and templates the formation of silica directly onto a conductive carbon paper electrode. Inclusion of CNTs and GOx into the reaction mixture results in a catalytic composite that becomes encapsulated as the silica forms[54]. The CNTs can act as nanowires within the silica matrix, essentially providing an electrical connection between the enzyme and the

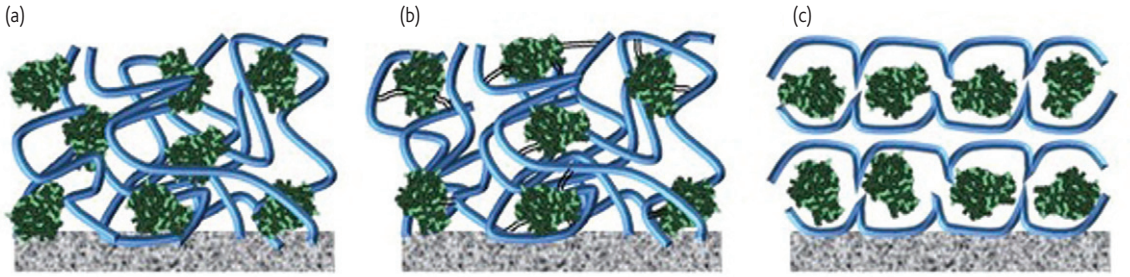

Fig. 2 Enzymes immobilized on an electrode surface via (a) physical adsorption to a polymer, (b) covalent attachment to a polymer (as shown by the black and white tethers), or (c) encapsulation in polymer micelles.

electrode and providing additional surface area for adsorption of active GOx as indicated by an increase in the electrochemically active surface area of a commercially available screen printed electrode to 3.7 cm^2. Similarly, enzymes can catalyze the reduction of metal salts to form discrete metal structures, such as gold nanoparticles. GOx, for example, will catalyze the reduction of gold (III) chloride with size-controllable formation of gold particles, into which the protein becomes entrained as the metal structure forms. The resulting GOx/gold composite retains the catalytic activity of the protein and DET is observed between the FAD cofactor and the electrode as shown voltammetrically via a peak at −0.44V vs Ag|AgCl; as such, a catalytic current is observed in response to glucose, which increases non-linearly from 5 mM to 25 mM glucose[55].

Direct interaction between the enzyme and electrode is a particular challenge for enzymes such as GOx where the cofactor is buried deep within the protein structure. This limitation can be overcome by anchoring the cofactor, FAD, directly to the electrode surface. The apoenzyme (enzyme without cofactor) is subsequently added and reforms around the anchored FAD; ensuring that the enzyme is in close communication with the electrode. The cofactor anchor is typically achieved by linking to gold nanoparticles or CNTs that serve as an electron bridge[56,57]. FAD, for example, can be linked to SWNT and used to position the apoenzyme of GOx; whereby the length of the CNTs directly influences the resulting electrical connectivity[58]. Similarly, Ivnitski *et al*., demonstrated the anchoring of GOx to CNT and observed DET between the active site of the enzyme and MWNT that were grown directly on a Toray® carbon electrode[59].

The introduction of CNT as a conductive material has provided a means to develop new conductive architectures, and a range of buckypaper (CNT paper), buckygel (CNT gels), and carbon nanofiber electrodes have since been reported that demonstrate a significant enhancement in electron transfer characteristics for both anodic and cathodic catalysts[60-63]. Cathodic oxygen reduction catalysts, for example can be immobilized to buckypaper, by simple physical adsorption[64], but preferential orientation is encouraged by using a bifunctional cross linking agent (1-pyrenebutanoic acid, succinimidyl ester; PBSE) that interacts with CNT via π-π stacking[62]. The tethering of laccase via PBSE results in stable cathodic currents and potential losses of < 0.1 V. Buckygels, in comparison incorporate ionic liquids and CNT in a composite material, into which NAD(P)H electrocatalysts (such as methylene green) can be added to help regenerate the enzyme cofactor $NAD(P)^+$ at moderate overpotentials[60].

Arguably one of the most significant contributions to improvements in biosensors and biological fuel cell development was the introduction of redox hydrogels, typically based on osmium or ruthenium complexes, into which enzymes could be effectively co-immobilized. This technical direction helped demonstrate the utility of biological fuel cells as implantable devices and with implications in diabetes management[65,66]. Enzyme catalysts are typically covalently bound to the hydrogel, and initial issues of long-term stability have been overcome by further anchoring the hydrogel to electrodes using surface carboxylates or amines[67,68]. Osmium-based redox hydrogels have been used for both anodic and cathodic electrodes. Although hydrogels are typically considered fragile, lifetimes greater than 14 days have been reported for enzymes in hydrogel matrices. Redox hydrogels have similarly been employed for microbial bioelectrocatalysis[69]. These types of combined immobilization and mediation strategies, however, have been much less common in microbial fuel cells due to the ability of the microbes to self-immobilize and grow nanowires/pilli to communicate directly with the electrode[20].

Materials for increased conductivity and surface area of electrodes

In the field of biological fuel cells, there are two types of conductivity that are important to performance, conductivity of the electrode and electrode components, and ionic conductivity between the electrodes. Ionic conductivity between the electrodes is typically separated into two types: ionic conductivity of the electrolyte solutions and ionic conductivity of the polymer electrolyte membrane separating the catholyte from the anolyte. Low conductivity results in large ohmic losses in biological fuel cell performance, so improving the conductivity of the overall system is important. Early in the development of traditional metal-catalyzed fuel cells, researchers designed fuel cells specifically to minimize the distance between electrodes. Many of these strategies have also been applied to biological fuel cells over the last decade, as the original H-cell setup has transitioned toward membrane-free strategies and membrane electrode assembly (MEA)-style biological fuel cells[70]. Fig. 3 shows the transition from H-cells to membrane-free electrochemical cells to MEA-style fuel cells. Interestingly, MEA-style fuel cells permit the incorporation of a bipolar plate design, which has also been demonstrated for microbial fuel cells[71]. H-cells typically have a minimum of distance of 1 cm between the anode and the cathode (although many cell designs have distances of greater than 10 cm) and typically the majority of that distance will be filled with low conductivity electrolyte solution (such as a biological buffer). The membrane-free strategy allows for closer electrode separation (typically less than 5 mm), and the separation is filled with a low conductivity electrolyte solution[72]. In comparison, MEA-style fuel cell designs typically have less than a 1 mm separation between the cathode and anode and the whole gap is a polymer electrolyte membrane[73,74].

From a materials perspective, researchers have focused on improving ionic conductivity by studying different polymer electrolyte membranes. Most polymer electrolyte membranes used in biological fuel cells are Nafion®, but most biological fuel cells operate at neutral pH, so a proton exchange membrane is non-ideal, because it has a higher resistance in potassium or sodium buffers at near neutral pH, than it would in the normal acidic environment of traditional fuel cells. For this reason, recent studies have addressed the development of alternative cation exchange membranes (i.e., Ultrex)[70] and alkaline exchange membranes[75]. They

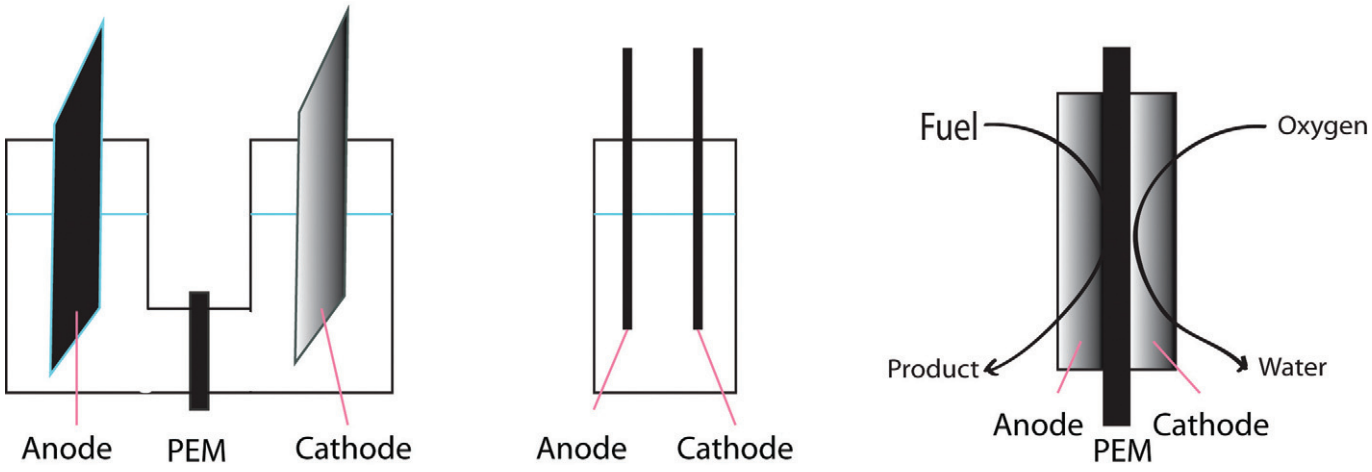

Fig. 3 Schematics showing the transition from the original biological fuel cell design (left figure, often called an H-cell) where the two electrode are submerged in two different solutions that are separated by a polymer electrolyte membrane (PEM), to the membrane-less biological fuel cell (center figure) where the two electrodes are submerged in the same solution and there is no separator/PEM, to the membrane electrode assembly (MEA) design (right figure) where the anode and cathode are in contact with the PEM.

have also studied bipolar membranes for microbial fuel cells[76]. As of yet, a polymer electrolyte membrane with high conductivity at near neutral pH or that can handle pH differences at the anode and cathode has yet to be achieved. The lack of polymer electrolyte membrane with high ionic conductivity at neutral pH and lack of a polymer electrolyte membrane that can effectively handle pH gradients between the anode and the cathode are major issues that will need to be addressed over the next period of materials research in biological fuel cells.

There is no easy way to compare differences in conductivity between electrochemical cells, in the absence of a standardized fuel cell design. The enzymatic biological fuel cell field typically does not determine ohmic resistances of cells, whereas this parameter is frequently reported for microbial fuel cell systems. There is, however, no standard practice for comparable reporting of this type of performance information. For instance, some reports document ohmic resistance per cubic meter of the bioelectrochemical system and other report ohmic resistance per square meter. In addition, resistance will be a function of the thickness as well as the properties of each membrane. Sleutels *et al*. began to address this bottleneck by directly comparing the differences between cation and anion exchange membranes. An internal resistance of 192 $m\Omega/m^2$ was reported for a biological fuel cell fabricated with an anion exchange membrane, versus 435 $m\Omega/m^2$ for a comparable cation exchange membrane-based cell[77].

The second issue associated with conductivity is the electrode or current collector conductivity, which is closely related to the development of high surface area materials. Since the volumetric catalytic activity of proteins, organelles, and living cells is low, it is important to have high surface area materials to load larger quantities of biocatalyst. The goal has been to maintain conductivity while increasing surface area. These high surface area materials focus on having a high surface area to volume or mass ratio. Most early biological fuel cell designs were glassy carbon, graphite, or reticulated vitreous carbon (RVC). The transition to higher surface area materials has included the incorporation of nanomaterials (discussed above) as well as the use of mesoporous carbon[19], carbon foams, buckypaper, and buckygels.

Hierarchical materials for improved mass transport

Most biological fuel cells currently reported in the literature are actually "bio-batteries"; they are either catalytic bio-electrodes immersed in a solution of the fuel or are meant to incorporate the fuel as a part of their design and as a result there is no continuous supply of fuel to the reactive layer. Lately, true "biological fuel cells" have been starting to emerge where the need to improve mass transport to and from the biocatalysts has become necessary[31,78,79]. Naturally, the design of materials for biological fuel cell applications followed the need to match the transport properties at the corresponding scale. At a macro-scale, the fluid flow needs to be accommodated and this results in design solutions with large void volumes (preferably more than 0.6), pore sizes in the range of 10 – 100 μm (and even up to 1 mm) and low tortuosity of the porous media. This scale of design is aimed to accommodate convective flow with rates below 1 cm^3/s. Such materials are preferred to have high electrical conductivity and are usually expected to provide the mechanical stability (rigidity) that is associated with building them into the biological fuel cell design as a structural component. Among the most widely used materials are different types of carbon (graphite) felts and carbon papers (Toray® paper being among the traditional sources). RVC and metal foams have also been introduced as a material of choice, particularly where 3D, bulk designs (such as cylindrical flow-through electrodes operating in a plug-flow regime) are being pursued[31,78,79].

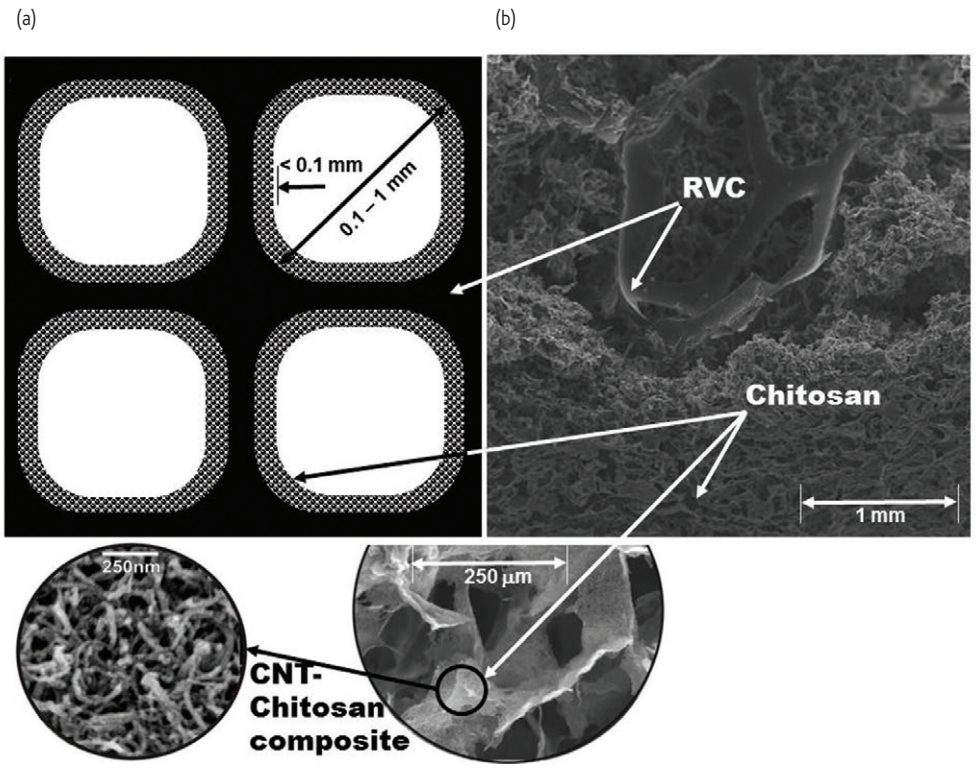

Fig 4 Schematic of a flow-through electrode material for a biological fuel cell that contains macropores in the reticulated vitreous carbon (RVC), micropores due to freeze drying of chitosan, and nanopores from the chitosan/CNT composite.

These macro-porous materials, however, cannot provide enough surface area for the immobilization of biocatalysts. Their intrinsic surface area is usually bellow 10 m^2/g (and often less that 1 m^2/g). This fact, combined with the practical enhancement of biocatalysts interactions with nano-materials (described above), calls for the integration of such materials with micro-porous or nano-scale, high-surface area materials. One example of such integration is the direct grafting of nano-materials onto open-pore structured substrates, such as CNT grown on Toray® paper[80, 54]. This macro-nano composite structure allows both substantial enzyme loading and promotes the desired nano-material/biocatalyst interactions. In a general case, however, such integration is difficult and much research has been focused on building hierarchically structured materials where all three levels of scale will be present: macro-scale porosity responsible for convective flow and fuel delivery; meso-scale architecture designed for the integration of materials properties; and the addition of nano-materials such as CNTs or gold nanoparticles, in the case of GDE[16], to smooth the progress of gaseous reactants (oxygen from ambient air) towards the reaction zone and micro-porous components[54,55,81]. The mesoporous component of such a composite matrix is usually responsible for interconnectivity and thus ensures the electrical conductivity of the matrix[82,83]. Fig. 4 represents both a schematic representation of an idealized material that embodies all three levels of porosity/structure along with an SEM microphotograph of an example of one such composite bioelectrode. In this case RVC is used as a conductive, macro-porous matrix, on the wall of its "foam-like" structure a conductive CNT/chitosan polymer composite layer is being formed that has its own porosity derived through a freeze-drying process and optimized for CNT content such as to demonstrate substantial conductivity and expose the "surface" of the CNT for immobilization of the biocatalysts. Such hierarchically-structured electrodes have been shown to be advantageous when used as enzyme anodes with immobilized oxidases or dehydrogenases or with microorganisms colonizing their inner space in microbial fuel cell anodes.

It is important to note that there are a variety of important factors to consider when designing a porous electrode for flow, including the hydrophobicity/hydrophilicity of the high surface area material to ensure wetting and avoid dead zones as well as the diffusional transport properties of the material. Paying close attention to these factors will result in improved biological fuel cell performance.

Conclusions

This review illustrates the strides in materials engineering that have contributed significant advancements in enzymatic and microbial biological fuel cell performance in the last decade. Further innovation is still needed, however, to fully harness the full potential of biological

fuel cells. These materials engineering advances have included the incorporation of high surface area materials to improve the loading of biocatalysts and to facilitate DET, materials for improved enzyme immobilization and stabilization, and the design of hierarchical material structures for advanced electrode design. Research is needed in the design of materials to improve the bio-nano interface to be more amenable to biocatalysts, as well as the production of structures that aid DET, so that high current density electrodes with long term stability can be realized. Secondly, a paradigm shift in the development of ion exchange membrane materials is needed to create membrane materials that are specifically designed for biological fuel cells rather than for typically highly acidic or highly alkaline environments of traditional fuel cells. mt

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